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The Chemical Examination of Thalosperma Gracile

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THE CHEMICAL EXAMINATION OF THALOSPERMA GRACILE

BY

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A. B. Eureka College, 1913

B.S. University of Illinois, 1914

THESIS

Submitted in Partial Fulfillment of the Requirements for the

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May 28, 1915.

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Clarence Barbre

ENTITLED **THE CHEMICAL EXAMINATION OF THE CONSTITUENTS OF THELESPERMA GRACILE**

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF **MASTER OF SCIENCE IN CHEMISTRY**

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THE CHEMICAL EXAMINATION OF THE CONSTITUENTS OF THELESPERMA GRACILE.

INTRODUCTION

From the earliest recorded writings of man we have the report of materials extracted from plants which are valuable for their curative power or ability to set up some nervous sensation. Many of the active agents in these preparations from plants have been isolated and identified as chemical compounds or definite mixtures of compounds with the result that some of them have been produced by synthesis. The laborious and costly collection and the still more difficult extraction processes have been eliminated. The records of extraction, identification and synthesis have not occupied a very large part of Chemical Literature for the reasons that the products were so valuable that the methods were not written and also, because of the difficulty of the work there has not been a large amount of reliable information to report. Methods for the preparation and estimation of single plant constituents, however, are voluminous compared with the methods for the accurate proximate analysis of plants.

The first article on the analysis of plants was published at the University of Prague by Rocheleder. It was later translated by Bastick and published in The British Journal of Pharmacy in 1860. It was revised by Maisch, the first American plant analyst, and published in the American Journal of Pharmacy in 1862. "An Introduction to the Chemical Analysis of Plants" by Wittstein was translat-

ed into English by Mueller and published at Melbourne in 1878.

During the time that Henry D. Parsons was a student under Prescott at the University of Michigan; he worked out and published "A Method for the Proximate Analysis of Plants" in the American Chemical Journal.

In 1884 Dragendorff published "A Qualitative and Quantitative Analysis of Plants" which was translated in 1886 by Greenish. The Wellcome Research Laboratory was established in London by H. D. Wellcome for the study of Tropical Medicine, Physiology, and Pharmacy. From this laboratory has come the best of all the methods proposed and about the only one that approached the accurate proximate analysis of plants. Until this time the best analyses gave only the percentages of ash, volatile matter, and the relative amounts of material extracted by various solvents. The Parson's scheme sought to separate the constituents into their respective classes such as alkaloids, glucosides, volatile oils, sugars, etcetera. By means of the Power's method some advance has been made. The pure compounds are identified, isolated and derivatives are made to prove the composition. The plan in brief is as follows:- Twenty-five kilos of the air-dry material are taken for analysis. It is ground in a mill until it will pass a sixty mesh sieve and a preliminary study made. A small portion (10 grams) is subjected to a test for alkaloids. The material is extracted with Prollius Mixture (Ethyl ether 325 parts by volume, ethyl alcohol 25 parts, Ammonium Hydroxide (sp.gr. 0.9) 10 parts.) The ethereal liquid is shaken with some dilute Hydrochloric acid, after which the aqueous solution is made alkaline with Ammonium Hydroxide, and the liberat-

ed alkaloid is extracted with ether. The ethereal liquid is evaporated leaving the alkaloid as a resinous or crystalline mass, which may be further purified by reformation and extraction of the Hydrochloride and subsequent treatment with Ammonia and ether.

The material is next examined for the presence of enzymes. About two hundred grams of the ground plant is macerated with water at ordinary temperature for several days, the liquid is expressed and filtered and twice the volume of alcohol is added. The precipitate produced in this way is tested with starch and other solutions for enzymatic change.

The moisture and ash are determined and the separation of the constituents into classes of organic compounds is begun. While the method of analysis does not attempt to isolate the identical compounds as they occur in the plant in every case, it does try to get them out with as gentle treatment and as little change as possible.

First, the ground material is thoroughly extracted with ninety-five per cent alcohol. This extract constitutes all of the material to be analyzed. The solvent is removed and data on the weight of the extract and its per cent of the whole weight is taken. The essential oil is now removed by mixing with water and distilling with steam. The material is now divided into two parts, water soluble and insoluble, by extraction with water. From this point, each part is extracted with various immiscible solvents in the order of their least solvent action. This is done so as to get as wide and sharp a separation as possible. The order of the solvents is determined on a twenty-five gram sample by extracting it successively

in a Soxhlet apparatus with various solvents and the weight of each extract taken. After the constituents are separated into classes, the members of each class are separated by the usual methods depending on their physical and chemical properties employed in the analysis of mixtures of organic compounds.

History of the Sample.

The material used in this investigation was collected in Mexico through the courtesy of Mr. Geo. Findlay and sent to Dr. G. D. Beal for analysis. With the plant came the statement that a water infusion of the plant was highly prized by the natives of the locality of collection for its use as an antidote in cases of snake bite.

Professor William Trelease, of the Botany Department of the University of Illinois, identified the plant as Thelesperma Gracile, Gray. No reference in the alkaloid literature could be found. The only information obtained was that it grows in the state of Kansas and in the south west. Thinking that the plant might contain an alkaloid, a preliminary study was made in 1913 by Mr. D. T. Englis. No alkaloid was found at this time nor subsequently.

The Object of the Investigation.

The purpose of the investigation was to test out the F. B. Power plan for the proximate analysis of plants and to determine, if possible, the chemical constituents of the plant Thelesperma Gracile.

EXPERIMENTAL

The material used in this investigation consisted of the whole air dry plant, except the roots, ground until it would pass a sixty mesh sieve. A small portion of the sample (10 grams) was subjected to the preliminary test for an alkaloid, when no indications were obtained of the presence of such a substance.

For the purpose of complete examination 2.24 kilos of the ground plant were completely extracted with hot alcohol when after the removal of the greater part of the solvent 120 grams (about 5.3%) of a viscid, dark green extract were obtained.

Distillation of the Extract with Steam and the Separation of the Essential Oil.

The entire amount of the above mentioned extract was mixed with water and distilled with steam for five hours. On extracting the distillate with ether and removing the solvent, a quantity of a pale yellow essential oil was obtained. There was about one cubic centimeter and since it was not a normal product its constants were not determined.

Non-volatile Constituents of the Extract.

After the removal of the essential oil as above described, there remained in the distillation flask a dark aqueous liquid (A), and a dark green oily resin (B). The resin was separated and, in order to wash it thoroughly, it was mixed with boiling water when an emulsion formed. The latter was shaken with petroleum ether

(B.P.40-50), after which the aqueous liquid which contained suspended matter was filtered, the filtrate being concentrated and added to the main portion of the aqueous liquid. The petroleum liquid was then evaporated and the residue mixed with the solid which had been collected on the filter, the total amount of the resin being thus brought together.

Examination of the Aqueous Liquid (A).

The whole of the aqueous liquid was first thoroughly extracted with ether (extract A1), then with petroleum ether (A2), then with chloroform (A3), and finally with iso-amyl alcohol (A4). These solvents removed some brown viscid material which gave a slight coloration with ferric chloride. Their respective solutions (A1-2-3-4) were each shaken successively with ammonium carbonate, sodium carbonate, potassium hydroxide, hydrochloric acid and chloroform; after which the alkaline solutions were acidified whereupon a dark green resinous material was thrown down which was filtered out and reserved. The acid solutions were concentrated and set aside. The acidified filtrate with the green resinous material were extracted with ether and various attempts were made to detect phenols; to separate them from the green coloring matter by the addition of various solvents to the ethereal solution. No satisfactory separations were obtained and the lightest colored precipitates could not be crystallized from alcohol of various concentrations in water. No green coloration with ferric chloride was observed and nothing definite could be obtained by this treatment.

After extracting the aqueous liquid with iso-amyl alcohol as above described, it was concentrated somewhat under diminished

pressure. (It was necessary to set this portion aside for some time, and although chloroform and xylol were added to prevent fermentation by the action of bacteria or moulds, some decomposition did occur which may have caused the following negative tests for sugars.) The concentrated solution was then treated with a slight excess of basic lead acetate solution, when a brown precipitate was produced. The latter was collected, washed, suspended in water, decomposed by hydrogen sulphide and the liquid filtered. The light reddish brown filtrate, on concentration under diminished pressure, yielded a dark colored product which was examined for tannin.

The liquid was heated with potassium hydroxide, acidified, extracted with ether and the ethereal liquid shaken out with aqueous ammonium carbonate. On acidifying and extracting with ether and the subsequent evaporation of the solvent, no evidences of tannin were obtained.

The filtrate from the precipitate produced by basic lead acetate was deprived of the lead by means of sodium sulphate and the lead sulphate filtered out. Alumina cream was added and the solution filtered and concentrated under diminished pressure. The concentrated solution failed to give any of the tests for sugars even after a portion had been hydrolyzed with dilute hydrochloric acid. The osazone test was run for a period of two hours with negative result.

Examination of the Resin (B).

The crude resinous material which had been separated from the aqueous liquid (A) was extracted successively in a flask under a reflux condenser with various solvents in the following order:

petroleum ether (B1), ethyl ether (B2), alcohol (B3).

Petroleum Extract of the Resin.

This extract, after the complete removal of the solvent formed a dark green, viscid mass. The entire extract was then introduced into a distillation flask, some water added and steam passed through the mixture for five hours. By this means a small amount of essential oil was eliminated which had not been removed by the distillation of the original alcoholic extract with steam. The contents of the distillation flask were saponified with alcoholic potassium hydroxide after the removal of a large part of the water. The resulting mixture was extracted with ether whereupon three layers separated; namely, the aqueous alkaline layer (B1a1), the ethereal layer (B1a3), and a relatively small amount of a sparingly soluble solid in suspension (B1a2).

Examination of the Volatile Acids.

The aqueous alkaline layer (B1a1) was acidified with ten per cent sulphuric acid, after removing the alcohol, and steam distilled. The distillate thus obtained was extracted with ether, the ether evaporated in a tared dish and the average molecular weight of the volatile acids determined. The average molecular weight was found to be 243.9. This corresponds to that of Pentadecylic acid which has a molecular weight of 240. This material was probably impure lauric acid. Since only 0.0387 grams were obtained, it was impossible to separate the mixture and determine the individual acids as was originally intended.

Examination of the Fixed Fatty Acids.

The residue (B1a1a) left after the distillation of the volatile acids was neutralized with sodium hydroxide and extracted with ether to remove any unsaponifiable matter. The aqueous liquid was then acidified and extracted with ether. During the evaporation the acids were lost by fire.

Examination of the Ethereal Layer (B1a3).

The ethereal layer obtained by extracting the hydrolyzed petroleum extract of the resin with ether as above described was dried and the solvent removed. A viscid dark yellow product was removed. It was found to consist for the most part of the solid hydrocarbon described below and a light yellow oil of indefinite composition. The weight of this product was about 0.5 grams.

Examination of the Suspended Solid (B1a2).

This solid was a neutral wax-like substance, and was easily obtained as a pure white solid free from chlorophyll by crystallization from alcohol diluted three to one with water. For the final purification, it was crystallized from acetic anhydride and washed with ether, and allowed to stand in a vacuum dessicator over potassium hydroxide. When prepared in this way, the solid melted sharply at 70.5 degrees, was friable and tasteless. On ignition on platinum foil it burned with a non-sooty flame and had a paraffin odor, leaving no residue. The solid contained no Nitrogen, Sulphur or Halogens. It was insoluble in the following liquids:- Water both hot and cold; Hydrochloric acid dilute and concentrated both cold and hot; Potassium hydroxide dilute and concentrated both

hot and cold. It was unaffected by hot concentrated sulphuric acid and was insoluble in the cold reagent. It was but slightly soluble in ether. The analysis of a 0.0940 gram sample gave the following results:-

	Per cent C	Per cent H	Total
Per cent found ----------	86.13	13.15	99.68
Per cent calculated for $(C_7 H_{14})_x$ -----	85.72	14.38	100.00

Ether Extract of the Resin (B2).

This extract, when free from the solvent, formed a dark green crisp mass. The ethereal solution was extracted with ammonium carbonate, sodium carbonate and potassium hydroxide. The alkaline liquids in each case were acidified with ten per cent sulphuric acid, when a very small quantity of green tarry material was precipitated. Since the combined precipitates amounted to less than one gram, further attempts to separate any acid substances were found impracticable.

The Alcoholic Extract of the Resin.

The residue from the above extractions was dissolved in alcohol and reserved for future investigation.

SUMMARY AND CONCLUSIONS

The material used in this investigation consisted of the dry stem and leaves of Thelesperma Gracile collected in Mexico about three years ago (1911 or 1912).

For the purpose of a complete examination 2.24 kilograms of the plant were used. This material was ground completely, extracted with hot alcohol, and the resulting extract distilled in a current of steam in order to remove the essential oil.

From the portion of the extract which was soluble in water no definite compounds were obtained, although effort was made to identify sugars and any tannin that might have been present. The sugar was probably small in amount and of this some was undoubtedly destroyed by fermentation. The tannin was not found for the reason that such a small sample was originally taken.

The portion of the alcoholic extract which was insoluble in water consisted of a green oily resin. From this resin which contained some occluded essential oil, the following substances were isolated:- (i) a hydrocarbon which has the formula $(C_7H_{14})_x$. The molecular weight was not determined because of the small amount of material available; (ii) a mixture of volatile fatty acids which were not separated; (iii) a mixture of fixed fatty acids which were lost by fire.

In consideration of the results of the foregoing investigation, the following conclusions are given. In the first place, the plant, as taken for analysis, contained no alkaloid, and therefore it is doubtful that the plant has any specific medicinal value.

In the second place, it seems highly important that at least 20 kilograms of a plant should be available before a complete analysis is undertaken for there is no doubt in the writer's mind that the method employed separates the constituents in such a way as to make their identification possible. Although the method is long, it has accomplished more in this direction than any other method.

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